THE ILLUMINATION OF THE EYE NECESSARY FOR DIFFERENT MELANOPHORIC RESPONSES OF FUNDULUS HETEROCLITUS [1]

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It has been established by previous investigations (Butcher, 1938) that the upper region of the retina of *Fundulus* is related to the paling of the body and the lower region to the darkening of the fish. During the course of these investigations many problems were encountered and left unsolved. Among them were: (1) Why does illumination of the lower region of the retina with a Mazda lamp cause most fishes to darken, but illumination of the upper region with a Mazda lamp induce only a few to pale? (2) How much of the regions have to be illuminated to elicit the related melanophoric responses? (3) To what extent does illumination of the upper region have to be eliminated in order that darkening can be induced by illuminating the lower region? (4) Is the paling response more easily elicited when light is entirely eliminated from the lower region?

The cause of a fish assuming a paler shade in a shaded white box than in a brightly lighted gray box was also investigated. It seemed that the assumption of the shade of the background by the fish might depend upon the ratio of the direct light coming from above and the reflected light from below which enters the eye as Sumner (1911), and Sumner and Keys (1929) have contended to be the case for the flounder.

The present investigations show that when a Mazda lamp is placed above fishes in a black dish, the image of the lamp falls upon enough retinal receptors in the lower region of the eye to induce darkening of the body, but when the same lamp is placed below fishes and its image falls upon the retinal receptors of the upper region, this image is not large enough to induce the paling response. Illumination of a large area of the upper region is, therefore, necessary to induce the paling response. Paling is also more easily elicited when only the upper region is illuminated. Illumination of the lower region induces darkening only when there is very little illumination to the upper region.

The melanophore response elicited by illuminating the lower region

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of the eye may be reduced by the simultaneous illumination of the upper region. For instance, illumination from above to the lower region of the eye causes darkening of the body when there is little reflected light from the bottom of a black dish to the upper region. If a gray bottom is used, a greater percentage of the light is reflected to the upper region of the eye, a greater inhibitory reaction is induced, and the degree of darkening of the body is reduced. If this gray back-

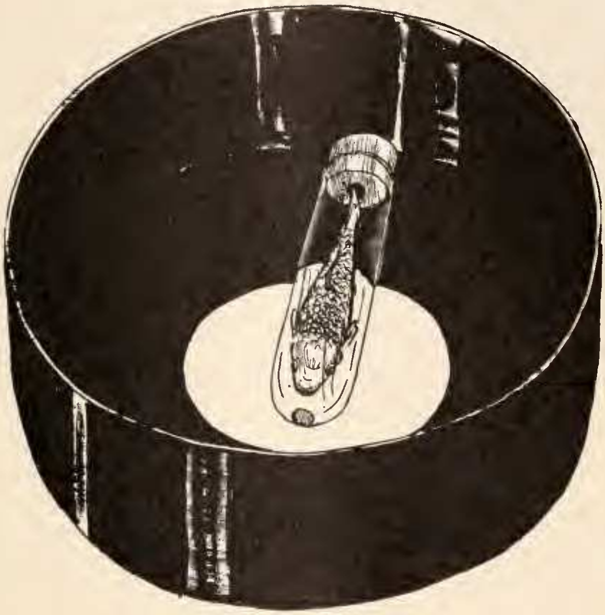


FIG. 1.

ground is more intensely illuminated from above, then the reflected light is greater to the upper region. The same ratio, however, persists between the direct and the reflected light, a proportional inhibitory effect is induced and the fish assumes the same shade as when the intensity of the direct light is lower.

The Conditions Affecting the Paling Response

The sides of crystallizing dishes, 20 cm. in diameter, were lined with black paper which reflected approximately 1 per cent of the light striking it. Fishes were placed in these dishes containing water 4 cm. deep, and the top of the dish was covered with black paper. When the fishes were illuminated through the glass bottom by a 60-watt, inside-frosted Mazda lamp, placed 18 cm. below the dish, a few of them became slightly pale and the rest assumed an intermediate shade. The fishes, in this instance, were receiving about 200 footcandles of illumination (determined by a Weston photronic illuminometer) from the lamp while the brightness of the lamp was approximately 58,000 footlamberts.[2]

If a piece of white paper or opal glass, as large as the bottom of the dish, were inserted between the source of illumination from the Mazda lamp and the bottom of the dish, the fishes readily paled even when the brightness below them was 1 footlambert or less. The image of the white bottom being larger than the image of the bulb alone fell on a great many more retinal receptors. Paling, therefore, depends mainly upon the size of the white area seen by the fishes.

TABLE I

Relation between body size and diameter of circle below fish necessary to induce paling.

Number examined	Length of body	Width of body at level of eyes	Diameter of circles and number paling			
			9 cm.	7 cm.	5 cm.	3 cm.
	mm.	*mm.*				
6	40–45	5–6		6	6	2
59	50–60	7–8	59	48	16	0
15	60–70	9–10	15	14	7	0
7	70–80	10	7	7	0	0

As a means of determining the size of the white area necessary for inducing paling of the body, fishes were enclosed in glass tubes (16 mm. inside diameter) which had small openings at both ends for the circulation of water. These tubes with the fish inside were placed over circles of white paper in such a way that the fish's head was above the center of the circle (Fig. 1). The circles were then either illuminated from above or from below.

To induce paling of fishes 50 mm. in length, circles 7 cm. in diameter were usually necessary (Table I). When the fishes were over 3 cm. circles, an intermediate shade was always assumed. Fishes paled equally as well when the circles were exposed to 4.5 footcandles as to 450 footcandles from above.

[2] The author is greatly indebted to Mr. Frank Benford of the General Electric Company, Schenectady, N. Y. for determining the brightnesses with a Luckiesh-Taylor Brightness-Meter.

Figure 2, which is drawn to scale, shows approximately the size of the image in the upper region of the eye when the fish was over the various circles. It is evident that a large area of retinal receptors must be stimulated before paling is induced. Images of 5 and 7 cm. circles, being nearly the same size (Fig. 2), caused only slight differences in the degree of paling. It might have been better to use a square tube to hold the fish, since a round tube probably acted as a cylindrical lens, and the fish did not get an image quite like the white circle and even illumination.

Fishes of various lengths were tested in tubes of the same size over circles of white paper. Since the body of a small fish covered less of the 3 cm. circle than did the body of a large fish, the small fish saw more of the circle (Figs. 3 and 4), and assumed a paler shade over the 3 cm. circle than the large fish (Table I).

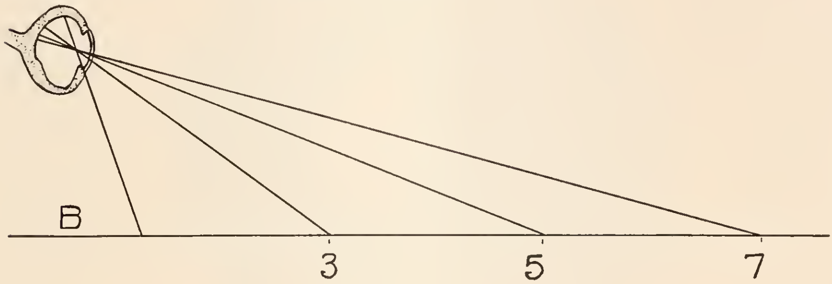
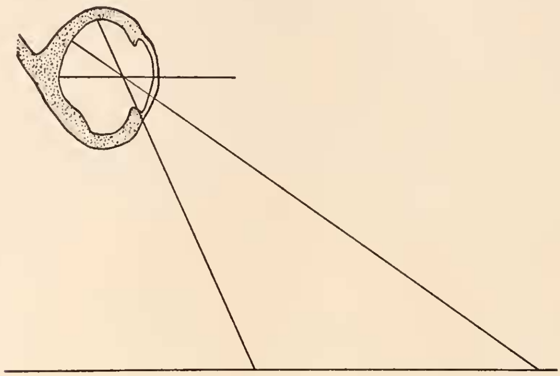


FIG. 2. Sizes of images in upper region of eye when fish was over circles with various diameters. Dorsal ventral diameter of eye—4 mm.; width of head—8 mm.; distance between eye and background—6 mm.; *B*., background; 3, 5, 7, boundaries of 3-, 5-, and 7-cm. circles.

Paling, therefore, depends greatly upon the size of the white area below the fish.

In previous experiments (Butcher, 1938) where blinders were used in covering the eyes, there was some evidence that illumination from above tended to inhibit the paling response or that paling was more easily elicited when the lower region of the eye was not simultaneously illuminated. There is no way of confirming this observation with a white background below and illumination from above because variation in the illumination from above causes a proportional variation in the reflected light from below. Likewise, if a white bottom is illuminated from below, causing the fish to pale, then illumination cannot be added from above in any way so that paling will not persist.

Whether or not light from above was inhibitory to the paling response was investigated in the following way. A circle of white paper which would induce paling when placed below a fish and illuminated from above (Fig. 1) was cut into halves. The fish in the glass tube was then placed over half of this circle in such a way that the axis of the fish corresponded with the diameter of the circle (Fig. 5). Fishes arranged in this manner failed to pale, because illumination of the



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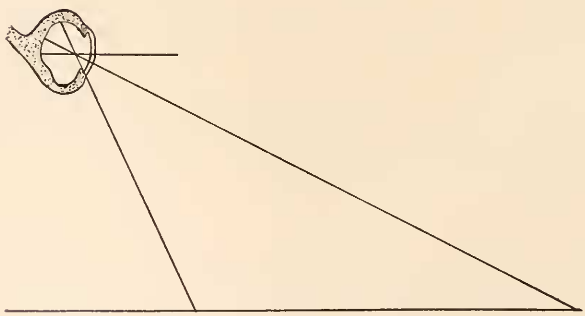

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FIGS. 3 AND 4. These figures illustrate that more of the 3 cm. circle is seen by the small fish than by the large fish. The image in the small fish covers approximately 15 per cent more of the upper region of the retina.

FIG. 3. Eye of fish 80 mm. long, and 10 mm. wide at eye level.

FIG. 4. Eye of fish 40 mm. long, and 6 mm. wide at eye level.

lower regions of both eyes was enough to inhibit any response elicited by the reflected light to the upper region of one eye. Even if the diameter of the circle was greatly increased, paling was not induced in most instances. When the eye which was not over the white semicircle was enucleated, the fish immediately paled, for now the inhibition resulting from illuminating the lower region of one eye was not enough to prevent the influence of the upper region of one eye. These experiments definitely showed that the paling response was more easily elicited when the lower regions of the eyes were not so intensely illuminated.

The Conditions Necessary for Inducing the Darkening Response

To determine the intensity of illumination of the eye necessary for inducing complete darkening of the body, fishes were placed in glass dishes lined with black paper. These dishes were about 20 cm. in diameter, 8 cm. deep, and contained water 4 cm. deep. A cylinder

FIG. 5. This figure shows how a fish was placed over a semi-circle so that the upper region of only one eye was illuminated by reflected light from below.

lined with a light-proof, black paper enclosed the dishes. The top of the cylinder was covered with opal glass, and a Mazda lamp, inside-frosted, was suspended above the cylinder as the source of illumination. The diffusing opal glass was 18 cm. from the surface of the water in the dish. Two small openings were made in the side of the cylinder. One was used for observing the fish and the other was large enough for transferring fish in and out of the black dish. The temperature of the water was kept at about 16° C. It was always ascertained if fish would assume both pale and dark shades before they were used for the experiments.

In investigating the effect of illuminating the lower region of the retina with different intensities three or four fishes were placed in the dish and allowed to remain for 20 minutes. Meanwhile, control fishes in other black dishes outside of the cylinder were being exposed to intensities from Mazda lamps which definitely induced maximum darkness. At the termination of 20 minutes, one studied the experimental fishes through the small hole in the side of the cylinder and observed their shades. In order to determine more definitely how many were completely dark and the correctness of the observations made in the experimental dish, the observer then viewed the control fishes, and without changing his field of vision, he quickly transferred an experimental animal into the control dish. This method involved only a few seconds and reduced the possibilities of error as much as any method used. Fishes were tested only once and then discarded.

When the intensity of illumination reaching the fishes was reduced to 2 footcandles, the majority of them failed to completely darken. Exposing 60 fishes to this intensity, 40 per cent of them became completely dark, 20 per cent darkish, and 40 per cent intermediate. The tables in the article by Brown (1936) show that an intensity of illumination of 1.75 footcandles caused complete dispersion of melanin in many *Ericymba buccata* Cope, the silver-mouthed minnow. Danielson (1938) reports that complete melanophore change appeared to occur at and above 1 footcandle in *Nocomis biguttatus* Kirtland. There are undoubtedly variations between different species and variations in threshold between different individuals. When the intensity was increased to 3.5 footcandles and 54 fish were tested, 45 became completely dark, and the other 9 had a darkish appearance.

Since it was necessary to stimulate a large area of receptors in the upper region of the retina in order to induce paling of the body, a few investigations were undertaken to determine the size of the source of light to the lower region necessary to cause the darkening response. In place of the opal glass covering of the cylinder, a black lid was substituted. This covering contained a central aperture, the size of which could be varied. When this aperture was 1.5 cm. in diameter and the fish were receiving 2 footcandles, about the same percentage (40 per cent) became maximally dark as when they received 2 footcandles through opal glass. Only 25 per cent, however, definitely assumed a maximum darkness when the diameter of the aperture was reduced to 1 cm. and they received an intensity of 2 footcandles.

Some *Funduli* thus become maximally dark when receiving an intensity of 2 footcandles from a source of light 1 cm. in diameter. The diameter of the image formed by a source of light 1 cm. in diameter and 18 cm. from the eye is only about .085 mm. or 85 micra. Whether or not more than 25 per cent of the fish will be induced to darken when receiving an intensity greater than 2 footcandles from a source 1 cm.

in diameter has not been determined. At least, a much smaller image induces darkening than the image necessary to elicit paling of the body. There are also undoubtedly individual differences in threshold.

Evidence that the Shade of the Fish Depends upon the Ratio between the Light from Above and the Light from Below Entering the Eye

Observations made by Sumner (1911), and Mast (1916) show that the shade of the flounder's body does not depend upon a visual comparison between its body surface and the background. It seems more probable from their experiments and those of Sumner and Keys (1929) that the ratio between the light coming from above and that reflected from below supplies the stimulus to the eye which enables the fish to assume a certain shade.

To learn if the ratio of light was responsible for the shade assumed by *Fundulus* it was first necessary to secure backgrounds which ranged in shade from white to black, to determine the response of the fish with each background, and the ratio of the direct to the reflected light in each instance. Various gray papers were used for these backgrounds and these were placed in the bottoms of large crystallizing dishes, the sides of which were lined with black paper. These crystallizing dishes were held with clamps about two feet from a table in a dark room. For illuminating the bottom of the dish, Mazda inside-frosted lamps were placed both above and below the dishes.

The response of the fish, the kind of bottom, and the brightness of the bottom when illuminated only from above are recorded in Table II. The responses of 15 fish were usually determined in each of these experiments. Gray 1, gray 2, and gray 3 were very close to neutral 6, 5, and 3, respectively, of the Munsell "Book of Color." [3]

The higher intensities induced about half of the fish in the dish with gray bottom 2 to pale. Reducing the brightness of the bottom to .1 footlambert when the intensity from above was .5 footcandle caused only a few to pale (Table II). Apparently the percentage of reflected light has to be greater than it is from bottom 2 (50 : 10) to induce all to pale.

If the fish assumes the shade of the background below because of the ratio of the light from above to the light from below entering the eye, then fish should pale over gray 3 when its brightness is increased by illumination from below, and a ratio is established which is known to induce paling of the body. To test this hypothesis the Mazda lamp, inside-frosted, was turned on under gray 3 while the intensity of

[3] "The atlas of the Munsell Color System," Munsell Color Company, Inc., Baltimore, Maryland.

illumination from above remained 5 footcandles. When the brightness of this paper was 3 footlamberts or a ratio (5 : 3) existed which induced paling over a white background, the fish, likewise, paled over this gray 3. With added illumination (400 footcandles) from above so that the ratio was 50 : 3.3, an intermediate shade was quickly assumed.

If either gray 1, 2, or 3 were illuminated only from below and their brightness was 1 footlambert (ratio in this instance is 0 : 1), all fish quickly paled. With no illumination from above fish could undoubtedly be induced to pale when the brightness of the bottom was much less than 1 footlambert for they have paled when the background was .2 footlambert in brightness and the intensity from above was .5 footcandle (Table II).

TABLE II

Light relations and shade of fish's body with different shades of paper below fish. *P.*, pale; *SP.*, slightly pale; *Int.*, intermediate.

Shade of paper below fish (sides of container black)	Intensity of light from above footcandles	Brightness of background below fish footlamberts	Ratio of light from above to brightness of background below	Shade assumed by fish
White	400	220	50 : 27	P
	10	5.5	50 : 27	P
	5	2.75	50 : 27	P
	.5	.275	50 : 27	P
Gray 1	400	160	50 : 20	P
	10	4	50 : 20	P
	5	2	50 : 20	P
	.5	.2	50 : 20	P
Gray 2	400	80	50 : 10	50% P, 50% SP
	10	2	50 : 10	50% P, 50% SP
	5	1	50 : 10	50% P, 50% SP
	.5	.1	50 : 10	20% P, 80% SP
Gray 3	400	24	50 : 3	Int.
	10	.6	50 : 3	Int.
	5	.3	50 : 3	Int.
	.5	.03	50 : 3	Int.

Some fish, therefore, paled when the ratio of direct to reflected light was 50 : 10. Fish failed to pale over a gray background below when lighted from above because this background did not reflect enough light in comparison to the light coming from above. If the gray background were illuminated from below so that its brightness was increased, then the fish paled.

Summary

Only a small area of the lower region of the eye of *Fundulus* needs be illuminated to induce a darkening of the body, since as little light as 2 footcandles coming from a source 1 cm. in diameter and 18 cm. above the fish elicits the darkening response. Darkening cannot be induced by illuminating the lower region when there is much illumination to the upper region of the eye.

For eliciting paling of the body, a large area of the upper region of the eye must be illuminated. This is shown by experiments with fish over circles. Regardless of the brightness of a circle 3 cm. in diameter beneath the fish, those 50 mm. in length failed to pale. When the illuminated circle was increased in size, fish paled readily. Paling, therefore, depends greatly upon the size of the white area seen by the fish. A Mazda lamp arranged below a fish so as to illuminate the upper region of the eye thus fails to induce paling because its image does not fall upon enough retinal receptors. Paling is more easily elicited when the lower region of the eye is not illuminated at the same time that the upper region is illuminated.

The ratio between the direct and the reflected light, known to exist with a white background below the fish, has been created with gray bottoms by illuminating them both from above and below. Gray backgrounds illuminated in this way have caused fish to pale readily. The shade assumed by *Funduli*, therefore, depends upon the ratio between the direct and the reflected light entering the eye.

As the percentage of reflected light to the upper region of the eye is increased, there is induced a proportional increase in the inhibitory reaction which causes a reduction in the degree of darkening of the fish.

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